PACIFIC BIOSCIENCES

	Application	Whole Genome Sequencing						RNA Sequencing		Metagenomics		Targeted Sequencing	
		De Novo Assembly - HiFi Reads	*De Novo* Assembly - for Low DNA Input	*De Novo* Assembly and Variant Detection - for Ultra-Low DNA Input	Microbial *De Novo* Assembly	Variant Detection	Structural Variation Detection	Iso-Seq Method	Single-Cell Iso-Seq	Full-length 16S rRNA Sequencing	Shotgun Metagenomic Profiling or Assembly	Amplicon Sequencing	No-Amp Targeted Sequencing
	Experimental Design												
	For Sequel II System Application-specific Bundle Kits and Part Numbers, see the Purchasing Guide												
Experimental Design	**With 1 SMRT Cell 8M you can:**	Produce reference quality assemblies for genomes up to 2 Gb	Produce reference quality assemblies for genomes up to 1 Gb Multiplex up to 2 small genomes on the Sequel II System	Produce reference quality assemblies for genomes up to 500 Mb	Sequence up to 48 microbes	With 2 SMRT Cells 8M, Call SNVs, InDels, and SVs in a 3 Gb genome	Call SVs for up to 2 samples with ~3 Gb genomes	Characterize alternative splicing/annotate a genome with full length transcripts	Characterize alternative splicing with full length transcripts up to 3M full length reads with cell barcode and UMI information	Multiplex up to 96 samples to provide strain level resolution	Generate near-complette assemblies of high-complexity sample(s) (e.g. gut microbiome)	Sequence 384 barcoded amplicons	Sequence 5 targeted regions in a multiplex of 20 samples
	Minimum Recommended Coverage	10- to 15-fold HiFi read coverage per haplotype	10- to 15-fold HiFi read coverage per haplotype	>30-fold HiFi read coverage per diploid sample for de novo assembly ≥15-fold HiFi read coverage for human variant detection	≥30-fold Unique Molecular Coverage (UMC) per microbial genome	≥15-fold HiFi read coverage of a human genome	5- to 25-fold UMC per diploid sample depending on study goals	One human transcriptome per SMRT Cell 8M	1,000 unique reads/ single cell for 3000 cells 10,000 unique reads/ single cell for 300 cells	8,000 HiFi reads / 16S sample	See Best practices guide	≥50-fold HiFi read coverage per target locus for variant detection 6,000-fold HiFi read coverage per target locus for minor variant detection (1% sensitivity)	≥100-fold HiFi read coverage per target locus
	Library Insert Size	15 - 20 kb	~15 kb	10 - 12 kb	10 - 15 kb	15 - 20 kb	>15 kb	<2 kb to >3 kb	<2 kb to >3 kb	1 - 2 kb	10 kb	500 bp - 15 kb	4-6 kb or larger
	Sample Preparation												
SMRTbell Template Preparation	**Procedure and Checklist Reference**	Preparing HiFi SMRTbell Libraries using SMRTbell Express Template Prep Kit 2.0	Preparing HiFi Libraries from Low DNA Input Using SMRTbell Express Template Prep Kit 2.0	Preparing HiFi SMRTbell Libraries from Ultra-Low DNA Input	Preparing Multiplexed Microbial Libraries Using SMRTbell Express Template Prep Kit 2.0	Preparing HiFi SMRTbell Libraries using SMRTbell Express Template Prep Kit 2.0	Preparing cDNA Libraries Using the SMRTbell Express Template Preparation Multiplexing SMRTbell Libraries using SMRTbell Express Template Prep Kit 2.0 for Structural Variation Detection	Iso-Seq Express Template Preparation for Sequel and Sequel II Systems	Preparing Single-Cell Iso-Seq Libraries Using SMRTbell Express Template Prep Kit 2.0	Amplification of Full-Length 16S Gene with Barcoded Primers for Multiplexed SMRTbell Library Preparation and Sequencing	Preparing 10 kb Library Using SMRTbell Express Template Prep Kit 2.0 for Metagenomics Shotgun Sequencing	Preparing SMRTbell Libraries using PacBio Barcoded Overhang Adapters for Multiplex SMRT Sequencing Preparing SMRTbell Libraries using PacBio Barcoded Universal Primers for Multiplex SMRT Sequencing	No-Amp-Targeted-Sequencing-Utilizing-the-CRISPR-Cas9-System
	Minimum Input Amount	15 µg	>400 ng per 1 Gb genome size (single-sample) >300 ng per 600 Mb genome size (2-plex)	5 - 20 ng per 500 Mb genome size	1 µg per microbe	15 µg	3 µg	300 ng total RNA for 1st Strand cDNA Synthesis	>160 ng cDNA AFTER reamplification	500 ng - 1 µg	1.5 µg	250-500 ng for 250-1000 bp 500-1000ng for 1-3 kb bp 1000-2000 ng for 3-10 kb 1500-3000 ng for 15kb	5 to 10 µg (represented by either a single sample or the total of multiple samples that will be multiplexed)
	Recommended Pacific Biosciences Template Prep Kit	Express TPK 2.0 + SMRTBell Enzyme Cleanup kit + Sequencing primer v2	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + SMRTBell Enzyme Cleanup kit + Sequencing primer v2	Express TPK 2.0 + Sequencing primer v2	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v2	Express TPK 2.0 +Sequencing primer v4	No-Amp Accessory Kit
	Multiplexing/SMRT Cell	N/A	N/A	N/A	Up to 48 microbes / SMRT Cell 8M Up to 16 microbes / SMRT Cell 1M	N/A	Up to 2 human samples/ SMRT Cell 8M N/A SMRT Cell 1M	The protocol supports up to 12 barcodes available.	Detects cell barcodes and UMIs	Up to 96 samples/ SMRT Cell 8M Up to 12 samples/ SMRT Cell 1M	Profile up to 4 communities/ SMRT Cell 8M Profile one community/ SMRT Cell 1M	Up to 1,000+ samples/ SMRT Cell 8M or SMRT Cell 1M	Up to 48 samples/ SMRT Cell 8M
	SMRT Sequencing with the Sequel II and Sequel IIe Systems: Loading and Pre-Extension Recommendations												
Sequencing Preparation and Yield Performance	**Pacific Biosciences Sequel II Binding Kit**	2.0	2.0	2.0	2.0	2.0	2.0	2.0 / 2.1*	2.0 / 2.1*	2.1	2.0	2.1: 500bp - 3,000 bp 2.0: ≥3,000 bp	2.0
	Sequel II Sequencing Plate	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
	Sequencing Mode	CCS	CCS	CCS	CLR	CCS	CLR	CCS	CCS	CCS	CCS	CCS	CCS
	Movie Collection Time	30 h	15 / 30 h	30 h	15 h	30 h	15 h	24 h	24 h	10 h	30 h	Insert Size-Dependent	≥10 h (30 h for repeat expansion targets)
	Notes							*Use Binding Kit 2.1 as default and 2.0 for long transcripts					
	SMRT Sequencing with the Sequel System: Loading and Pre-Extension Recommendations												
Sequencing Preparation and Yield Performance	**Pacific Biosciences Sequel Binding kit**	3.0	3.0	NA	3.0	3.0	3.0	3.0	3.0	3.0		3.0	3.0
	Sequel Sequencing Plate	3.0	3.0	NA	3.0	3.0	3.0	3.0	3.0	3.0		3.0	3.0
	Sequencing Mode	CCS	CCS	NA	CLR	CCS	CLR	CCS	CCS	CCS		CCS	CCS
	Movie Collection Time	20 h	20 h	NA	10 h	20 h	10 h	20 h	20 h	10 h		Insert Size-Dependent	≥10 h (20 h for repeat expansion targets)
	Notes			NA									
	Data Analysis Tools Available Through SMRT Link, PacBio DevNet and Other Compatible Software Tools												
Other Compatible Software Tools (Contact FAS for Support)	**SMRT Analysis GUI Applications**	Genome Assembly powered by IPA	Genome Assembly powered by IPA	Mark PCR Duplicates, Trim gDNA Amplification Adapters, Structural Variant Calling, Genome Assembly powered by IPA	Demultiplex Barcodes followed by Microbial Assembly analysis	CCS with Mapping	Structural Variant Calling	Iso-Seq	Iso-Seq	CCS	CCS	CCS or Long Amplicon Analysis	Demultiplex Barcodes followed by CCS with Mapping analysis
	PacBio Devnet Tools			lima, pbmarkdups, pbsv									Repeat Analysis Tools
	Other Analysis Tools	hifiasm, HiCanu	hifiasm, HiCanu	hifiasm, HiCanu		Recommend: Google Deep Variant			Github Tools			PacBio Amplicon Analysis (pbaa)	

Read lengths, reads/data per SMRT Cell and other sequencing performance results vary based on sample quality/type and insert size

PacBio Glossary of Terms

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